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Colony kin relatedness and male production in *Dolichovespula arenaria*
Thesis directed by Professor Michael D. Breed

The number of times a female social insect mates has important implications for the evolution and maintenance of sociality. My study focuses on queen mating frequency in a social wasp, *Dolichovespula arenaria* (Order: Hymenoptera, Family: Vespidae, Subfamily: Vespinae). Queen mating frequencies are known for only a few species of vespine wasps; adding data for additional species will help to establish trends in social evolution within this clade. Colonies of *D. arenaria* were collected in the subalpine zone of the Front Range of the Colorado Rocky Mountains in 2002 and 2003. Worker, queen, and male genotypes were analyzed using three DNA microsatellite loci Rufa 5, 13 and 15 (Thorén *et al.* 1995, Foster *et al.* 2001). Worker relatedness ranged from 0.57 to 0.83 within each of the seven colonies sampled with a mean relatedness of 0.77. Five of the seven colonies supported the hypothesis of single mating by queens. In one colony two and in one colony three matings were likely. Effective paternity in these two colonies was 1.48 and 1.91, respectively. Four colonies sampled contained males that were assigned as worker or queen progeny using both the exclusion and maximum likelihood methods; each method yielded similar results. In two of these colonies, all males were likely progeny of the queen. In the other two colonies worker produced 8 to 24% of the males. Overall 91.5% of the males in the population were likely progeny of the queen. These patterns are consistent with published studies of vespine wasps.

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COLONY KIN RELATEDNESS AND MALE PRODUCTION
IN *DOLICHOVESPULA ARENARIA*

by

BRIAN JAMES FREIBURGER

B.A. United States Air Force Academy, 1994

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Introduction

Why has eusociality evolved and why does it persist? This question puzzled Darwin (Darwin 1859) and remains an important biological question today. One factor that enhances the likelihood of the evolution and maintenance of eusociality is haplodiploidy. Hamilton (1964) recognized the high coefficient of relatedness in haplodiploid species and argued that eusociality could evolve through the benefits of inclusive fitness (one's direct fitness added to indirect reproductive success gain through shared genes of close relatives and their offspring) and the process of kin selection. A single mating by the queen (monandry) of a colony is a key component of Hamilton's inclusive fitness and kin selection theory because it yields higher worker-worker relatedness values. Also based upon a single mating, subsequent theories have predicted a queen-worker conflict over male production. Over the past four decades many studies of social organisms aimed to quantify the number of matings (or effective paternity) and subsequently determine the winner of the queen-worker conflict over male production in order to find evidence of Hamilton's theory of the evolution and maintenance of eusociality in nature. In my study of *Dolichvespula arenaria*, I calculated worker-worker relatedness, effective paternity and determined the winner of the conflict over male production. These results contribute to a better understanding of the evolution of social wasps and the behavioral dynamics that maintain this social species from year to year.

The Study Species

Hymenoptera is the third largest order of insects and is distributed worldwide. There are roughly 26 hymenopteran families and nearly 130,000 described species (Romoser and Stoffolano 1998). The family Vespidae consists of 5 genera, including the closely related *Dolichovespula* and *Vespula* -- commonly known as the yellow jackets and hornets (Romoser and Stoffolano 1998). In the 1970's the genus *Vespula*, which contained 35-40 species, was split into two genera based on more than twelve derived traits, *Vespula* and *Dolichovespula* (Greene 1991). *Vespula* now consists of "short cheeked" wasps (these have a narrow malar space) which maintain subterranean nests. While *Dolichovespula* have a long malar space ("long cheeked" wasps) and primarily build aerial nests (Greene 1991). Although some contention still exists whether or not this split was warranted, it is generally followed.

Social biologists further divide *Dolichovespula* and *Vespula* by the size of the nest; the nest represents social development and ecological niche without strictly reflecting phylogenetic groupings (Greene 1991). When classified by large or small nest size, two-thirds of yellow jacket species construct small nests. All species of *Dolichovespula* are members of the small-nest group, whereas most *Vespula* species maintain larger nests. Mature small nests usually contain fewer than 4,000 cells, but commonly only a few hundred cells and maximum worker populations of fewer than 800 individuals (Greene 1991). Most species within the small-nest group (*D. arenaria* is the exception) rear workers on only one comb and continuously expand this comb from the nucleus of cells constructed by the foundress. *D. arenaria* colonies expand the first worker comb throughout the season, but they also build a

complete second and sometimes third comb solely dedicated to rearing workers (Greene *et al.* 1976). This large work force inherent to *D. arenaria* enables this species to be among the most productive of small aerial nesters (Greene 1991). Each of the subsequent combs consists of larger cells that allow for the rearing of larger reproductives (fertile males and potential queens).

D. arenaria has a holarctic, boreal habitat distribution including the Rocky Mountains and maintains an annual life cycle with a relatively short colony life (Akre *et al.* 1980). Colonies live 15-17 weeks, which is typical of small aerial nesters, but colony longevity can vary with elevation and latitude. In North America, nest initiation typically begins in mid-May and nests reach complete decline by mid-September (Greene *et al.* 1976). Nests are constructed by maxillating wood pulp to produce the relatively strong, flexible paper used for both the comb and the external envelopes. As with all annual nesting yellow jackets, *D. arenaria's* persistence from year to year is dependent upon the female reproductives (potential queens) that emerge from the nest late in the season, mate and then seek out an over-wintering site. The over-wintering site needs to protect the future queen from both predation and long periods of freezing temperatures. These sites are usually rotting logs, trees, man-made structures or rock crevices. Typically, the production of reproductives (males and females) marks the start of the decline of the colony (Greene 1991). As winter sets in, all other colony members die and the nest is not used again. Each spring the individual female survivors (foundresses) from the previous season's nests begin the cycle again as they select a site and start to build a nest.

A foundress's nest site selection, whether aerial or subterranean, is key to the survival of the colony that will follow. Aerial nests are much more exposed to the environment, such as wind, hail, and temperature extremes; in addition, bird predation tends to be higher in these nests than those below ground (Green 1991). Subterranean nests are less exposed and can more readily maintain a microclimate suitable for brood survival, although they require a greater investment of energy to prepare and expand the cavity through the season. Additionally, subterranean nests are at risk from medium to large mammals that can completely destroy a nest, e.g. skunks and raccoons (Greene 1991). However, *D. arenaria* are renowned for nest defense, especially when approached by humans. Not only do they have a venomous sting, which can be used repeatedly, but also they have the ability to spray venom out of the stinger.

Haplodiploidy, Mating Frequency, Male Production, and the Evolution of Eusociality

Among animals, eusociality has evolved at least twelve times, once in a diplodiploid clade, the termites, and more than eleven times in haplodiploid (haploid males and diploid females) clades (Trivers and Hare 1976, Wilson 2000). One other instance of eusociality has recently been discovered in the Order Thysanoptera, a haplodiploid thrip (Crespi 1992). All Hymenoptera (ants, bees and wasps) have haplodiploid sex determination. When attempting to explain the evolution of eusociality, Hamilton (1964) naturally looked to the Hymenoptera as it contains most of the eusocial species.

Our understanding of eusocial insects was revolutionized by Hamilton's (1964) insights. He argued that altruistic behavior was more favored in haplodiploid species because of asymmetric genetic relatedness (Table 1). Hamilton's rule for altruistic behavior, $C/B < r$, where C is the cost and B is the benefit to the initiator (a) of an altruistic act and r is the relatedness coefficient between the two individuals (a and b), helps to explain the effect of these asymmetries. According to this rule, the relatedness of the individual (b) that benefits from the altruistic act to the initiator of the act (a) must be higher than the cost/benefit ratio this act imposes. When weighing the costs and benefits of a particular altruistic behavior in the singly mated Hymenoptera, the benefit can be lower because the relatedness is higher between sisters in haplodiploid species. Therefore, altruism is more favored in haplodiploid organisms.

Table 1. Hamilton's (1964) Asymmetric Genetic Relatedness of Singly Mated Haplodiploid Species

Relation	Female	Male
Mother	1/2	1
Father	1	N/A
Full Sister	3/4	1/2
Brother	1/4	1/2
Daughter	1/2	1
Son	1/2	N/A

Hamilton (1964) expanded the idea of classical fitness, which defines an individual's reproductive success as the sum of its offspring, by introducing the concept of inclusive fitness. He defined inclusive fitness as the number of an individual's offspring added to the number of its mother's and sibling's offspring, divided by the average number of offspring per individual within the population.

$$\text{inclusive fitness} = \frac{\begin{array}{l} \text{(own contribution} \\ \text{+ contribution of relatives)} \end{array}}{\begin{array}{l} \text{average contribution} \\ \text{of the population} \end{array}}$$

Inclusive fitness considers the genetic relatedness between and among generations, thus considering an individual's ability to pass on genes not only through direct offspring but via the genes of its close relatives.

By comparing degrees of relatedness in Table 1, Hamilton (1964) predicted the following outcomes, as it applies to social behavioral traits of Hymenoptera: (1) A female is more related to her full sisters than she is to her own children. She may therefore evolve an inclination to work in the maternal nest rather than start her own. (2) A male is more related to his daughters than to his siblings and therefore is not expected to evolve worker instincts. (3) A female is more related to her own sons than to her brothers and thus should be reluctant to provide for brothers and may instead lay her own unfertilized eggs to produce sons.

Outcomes one and three are most germane to my work, and can be combined and summarized as follows: a female does not actually need to produce any offspring to achieve reproductive success because she shares three-quarters of the same genes

with her sisters, some of whom will become queens of their own colonies. For these outcomes to be supported, queens must mate only once; if a queen mates more than once, then the inclusive fitness advantages of being a worker are reduced as worker-worker relatedness is reduced.

Trivers and Hare (1976) further explored the ideas of asymmetric relatedness and inclusive fitness. They hypothesized that workers should lay eggs (all of which would be haploid males), because workers are more related to their own sons than to their brothers. The daughter's ability to exploit her situation within a colony is the basis for conflict with the queen over laying male-destined eggs. Thus, the potential for conflict over male production in social Hymenoptera is widespread (Hamilton 1964, Woyciechowki and Lomnicki 1987, Foster *et al.* 2001). The resolution of this conflict within a colony is important to social cooperation and ultimately determines who achieves a higher inclusive fitness and, more specifically, who transmits her genes via male progeny into the next generation.

Trivers and Hare (1976) also noted that Hamilton (1964) modeled singly mated (monoandry) Hymenoptera queens but failed to describe intracolony relatedness when the queen mates multiply (polyandry). If the queen mates more than once, the result is a lower degree of genetic relatedness within the hive as compared to Hamilton's original predictions (Table 1). Therefore workers (females) would no longer be three-quarters related, on average, to their sisters (other workers). Under such circumstances, the high degree of relatedness and the strong social dynamics predicted by Hamilton (1964) would decrease.

If eusocial Hymenoptera are routinely polyandrous, the benefits of polyandry must outweigh those of monandry. Strassmann (2001) lists seven theories which attempt to explain why females (i.e. future queens) may be polyandrous: 1) the need for more sperm, 2) the potential for material gains (e.g. nutritious nuptial gifts or spermatophores), 3) the females' energetic costs associated with avoiding males, 4) the difficulties of identifying the best male, 5) possible genetic incompatibility, 6) the females' limited access to the best possible male, and 7) the advantages of a genetically diverse brood.

Strassmann (2001) argues that not all of these theories are applicable to Hymenoptera and some can be quickly ruled out. In Hymenoptera, males provide no spermatophores or territories and generally do not care for their offspring (theory two). In general, males cannot force a female to have sex, because she must move aside her stinger to copulate; this also reduces the female's cost of avoiding males (theory three). With males providing little to no care for offspring, there is no "best male" for parenting purposes (theory four). Finally, Hymenoptera have not demonstrated the post-copulatory discrimination mechanisms required to avoid genetic incompatibility nor do they have the ability to assess the best possible male (theories five and six).

Therefore, of the initial seven theories presented, only two are relevant to the reproductive biology of Hymenoptera; the queen's need for more sperm (theory one) and the advantages of genetically diverse brood (theory seven) (Strassmann 2001). Given the sheer number of fertilized eggs a female may lay over her lifetime, it is conceivable that she may require more sperm than one male can provide (theory one).

As for theory seven, the advantages of genetic diversity can be broken down into four positive effects:

(1) Increased resistance to disease (Schmid-Hempel and Crozier 1999).

There is good evidence that different genotypes vary in their susceptibilities to diseases; genetic variability not only decreases the number of individuals killed by parasites, but also slows the spreading rate through the colony (Jennions and Petrie 2000).

(2) Better facilitation of task specialization (Fewell and Page 1993). In honeybees and ants, offspring from different fathers tend to specialize at different colony tasks, possibly leading to higher efficiency and greater reproductive output (Fewell and Page 1993). However, there could be a cost as well: if half-siblings can detect each other it may lead to a bias of rearing only full-siblings, thereby reducing cooperation within the hive and decreasing reproductive output (Jennions and Petrie 2000).

(3) Decreased production of sterile diploid males (Strassmann 2001).

Increased matings, if effective, reduce the possibilities of producing sterile diploid males (Strassmann 2001). Complementary sex determination (CSD) occurs in Hymenoptera, meaning that if offspring are homozygous at any CSD locus the result is a sterile diploid male. Sterile diploid males reduce the effective workforce in the colony, which can reduce the colony growth rate and lower investment in reproductives (fertile males and potential queens) (Strassmann 2001).

(4) Decreased queen-worker conflict over control of sex-ratio and male production (Jennions and Petrie 2000). Queens may benefit from multiple matings

because they reduce worker-worker relatedness and thus cause workers to invest equally in each sex. Ultimately, multiple mating is predicted to decrease selection on the workers to prevent the queen from laying male destined eggs. In terms of worker inclusive fitness, the impact of this can be quite large, because these males mate with new queens who establish new hives. If the queen wins this conflict with her workers, she essentially gains thousands of grandchildren for each new colony established. However, this theory was not supported in Fjerdingstad et al.'s (2002) study of a European ant. They found no evidence that the number of matings by the queen affected the worker's behavior relating to sex-ratio. In general, more experimental work on Hymenoptera is needed before we can draw definite conclusions about the benefits of polyandry.

The mere existence of polyandry in Hymenoptera implies that at least some of these benefits outweigh the costs of diminished intracolony relatedness. The possibility of multiple mating spurred investigators to measure the actual number of times a queen mates for hundreds of species of eusocial Hymenoptera. Early studies were based on observations of the number of copulations or estimates of the number of sperm in the queen's spermatheca. The results of these studies over approximately 15 years led to the general consensus that many species of Hymenoptera have polyandrous queens (Page 1986). Of the 66 Hymenoptera species considered in Page's review (1986), 45 were thought to be polyandrous.

During the late 1980's, improved data collection methods led to an improved estimate of the prevalence of multiple mating in Hymenoptera. Allozyme marker technology was applied to putative polyandrous Hymenoptera (Strassmann *et al.*

1989) and by the early 1990's, DNA fingerprinting techniques were developed for paternity testing (Blanchetota 1991). Shortly thereafter microsatellites were developed for numerous insect species (Thorén 1995); this greatly enhanced scientist's ability to more accurately determine paternity and the prevalence of multiple mating.

Boomsma and Ratnieks (1996) compared results from various techniques (observed copulations, estimates of sperm numbers in males and females, and allozyme marker data) used to determine paternity in Hymenoptera. They found that many previous findings over-estimated the paternity frequency; they calculated paternity frequency of 19 ant species to be 1.16 via allozyme analyses. Boomsma and Ratnieks (1996) define paternity frequency as the number of times a queen mates regardless of proportional contribution to offspring by each male. A paternity frequency less than two effectively indicates a single mating, as worker-worker relatedness decreases only slightly. Thus, allozyme markers more accurately count the males that actually father a queen's offspring. Allozyme data on bees and wasps show a similar trend (Boomsma and Ratnieks 1996). Because only two species of ants show a paternity frequency higher than two, Boomsma and Ratnieks concluded that within Hymenoptera, paternity frequencies above two are restricted to three phylogenetically isolated and highly eusocial taxon: leafcutter ants (*Atta*), honey bees (*Apis*), and some eusocial wasps (*Vespula*). If low paternity frequencies (< 2) reflect a high degree of worker-worker relatedness (Table 1) and high paternity frequencies (> 2) reflect a lower degree of worker-worker relatedness, Boomsma and Ratnieks'

(1996) results for Hymenoptera support Hamilton's (1964) original theory of relatedness and eusocial evolution.

These results, combined with the advent of even more accurate microsatellite techniques, have led to extensive paternity research across a variety of hymenopterous taxa. The following is a summary of the recent effective paternity research conducted with microsatellites on a variety of bee and wasp species and ultimately ending with current work on my focal species *D. arenaria*.

In honey bees, *Apis spp.*, Tarpy and Nielsen (2002) reviewed 24 studies that estimated paternity frequencies of naturally mated queens using molecular techniques. Although the extremity of honey bee polyandry may be exaggerated, the average effective paternity is still quite high relative to other Hymenoptera species. Specifically, 80.5% of colonies tested had an effective paternity higher than 5.0, with 53.7% being higher than 10.0. Given this high degree of polyandry and thus low worker-worker relatedness, honey bee workers' police each others egg laying behavior, allowing the queen to produce nearly all of the males (Woyciechowki and Lomnicki 1987, Ratnieks and Visscher 1989). In this situation, the workers are more related to the queen's sons than their sister's (other workers) sons. As honey bees tend to practice extreme polyandry, Tarpy and Nielsen (2002) suggest that future studies on honey bees should focus on variation of effective paternity and its impact on the evolutionary benefits of polyandry.

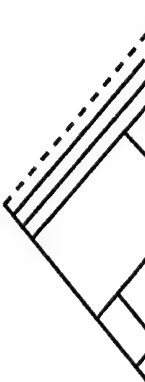
In stingless bee species effective paternity is well documented to approximate 1.0. However, the winner of the ensuing conflict between the queen and the workers over male production seems to be highly variable – ranging from 100% queen

production of males to 100% worker production of males (Tóth *et al.* 2002a, Tóth *et al.* 2002b, Paxton *et al.* 2003, Tóth *et al.* 2003). Collectively these studies represent nine species with no prevailing trends. Therefore more species must be sampled to better develop the overall picture for stingless bees.

Brown *et al.* (2003) looked at a species of bumble bee that was previously thought to be polyandrous (Schmid-Hempel and Schmid-Hempel 2000) using microsatellites and discovered an effective paternity of 1.0. In this case, the workers produced over 20% of the males (Brown *et al.* 2003). In another highly studied taxon, the family Halictidae (or sweat bees) Paxton *et al.* (2002), found an effective paternity of 1.29 across 18 naturally occurring colonies, again indicating a prevalence of single mating.

The research of eusocial wasps is most pertinent to my study, however no clear trends toward polyandry or monandry currently exist. According to Seppä *et al.* (2002) and Strassmann *et al.* (2003) at least two species of *Polistes* have effective paternities of 1.0. Further, Strassmann *et al.* (2003) found that when the queen was present she maintained control over male production but in those nests that lacked a queen upon collection the workers had produced all of the males. Studies of paternity in the subfamilies Polistinae and Vespinae have been summarized by Foster and Ratnieks (2001) (Figure 1).

Figure 1. Paternity frequency, worker reproduction and ovary development and frequency of queenless reproductive-stage colonies in Vespinae wasps. References: (1) Field *et al.* 1998; (2) Peters *et al.* 1995; (3) Foster *et al.* 1999; (4) Foster *et al.* 2000; (5) Foster (unpubl.); (6) Foster *et al.* 2001; (7) Ratnieks and Boomsma (unpubl.); (8) Goodisman *et al.* (unpubl.); (9) Ross 1985; (10) Ross 1985; (11) Foster and Ratnieks 2001a; (12) Thorén *et al.* 1995; (13) Spradbery 1971. Figure adapted from Foster and Ratnieks 2001a.

Clade	Species	Paternity		% males sons of workers	% workers with active ovaries	% reproductive nests		Refs.
		Effective	Range (n)			Queenless (n)		
	Polistinae	<i>Polistes</i>	< 1.05	1-2	-	-	-	1,2
		<i>Vespa crabro</i>	1.11	1-3 (14)	0	1	14 (14)	3,4
		<i>Provespa anomala</i>	1.00	1 (1)	-	-	-	5
		<i>D. maculata</i> grp	1.00	1 (10)	21	-	74 (19)	6
		<i>media</i>	1.08	1-2 (10)	7	5	32 (19)	6
		<i>D. norwegica</i> grp	1.09	1-4 (20)	17	4	54 (13)	7
		<i>arenaria</i>	1.15	1-2 (10)	10	7	36 (14)	6
		<i>sylvestris</i>	1.08	1-3 (10)	3	10	86 (14)	6
		<i>norwegica</i>	1.35	1-3 (10)	35	11	20 (10)	6
		<i>saxonica</i>	2.35	1-7 (55)	-	0.6	0 (25)	8,9,13
		<i>V. vulgaris</i> grp	7.14	- (30)	0	2	-	9,10
		<i>germanica</i>	1.90	1-4 (17)	0	0.4	6 (50)	11,13
		<i>maculifrons</i>	-	-	-	-	-	-
		<i>vulgaris</i>	-	-	-	-	-	-
	<i>V. koreensis</i> grp	no data	-	-	-	-	-	
	<i>V. rufa</i> grp	<i>rufa</i>	-	> 1 (1)	-	-	-	12
	<i>V. squamosa</i> grp	<i>squamosa</i>	3.33	- (17)	0	-	-	10

The majority of species present in figure 1 are monandrous, although even within this relatively small data set the exceptions of *V. germanica*, *maculifrons* and *squamosa* are sufficient to withhold any general conclusion about wasp paternity. Foster and Ratnieks (2001) allude to a study of *D. arenaria* in England, but have not published any results. Given the geographic separation of the North American population, which I am working with, and the European population studied by Ratnieks and Boomsma (unpublished) my study should reveal interesting comparisons and provide published results for the scientific community.

My work with the social wasp, *Dolichovespula arenaria*, is stimulated by theories prompted by Hamilton's relatedness theory. His premise of relatedness paved the way for further research in social insect dynamics to include the intrigue

over potential multiple matings by the foundress queen. In this study, I determined the effective number of times a queen mates and its consequences on the social structure and social behavior of the colony.

Specific Aims

1) I calculated the effective paternity using DNA microsatellites to genotype workers within colonies of *D. arenaria*. If, as expected based on previous research, I find a low effective paternity, then a conflict between the queen and workers over male production should exist (Trivers and Hare 1976, Foster *et al.* 2001). 2) Using the same techniques, I then determined who, either the queen or workers, produced the majority of the males. I predicted low worker male production in the presence of the maternal queen and complete worker male production in the absence of the maternal queen. The winner of this conflict ultimately has a higher inclusive fitness because these males inseminate the foundress queens of next year's colonies, thereby passing on the winner's genes and potentially impacting the evolution and maintenance of eusociality in wasps.

Material and methods

Species collection

Colony collection was a two pronged effort, consisting of artificial nesting boxes and opportunistic natural nest collection. My field site was located at the University of Colorado Mountain Research Station, where 200 artificial ground-nesting boxes were placed in the field during late May 2002. Nesting boxes were constructed of 0.95 cm plywood with 20x15x15 cm dimensions. There was a single

1.6 cm entry hole for the wasps and a slide off lid to allow access for the researcher. Boxes were placed in shaded locations under bushes and trees. These boxes were monitored throughout the summer months for wasp activity as well as for nest mortality if prior to the collection of all the boxes in early September.

In addition to the ground-level boxes, 53 aerial nesting boxes were placed in the field in May of 2003. These nest boxes were constructed of 0.95 cm plywood as well, but with 20x30x38 cm dimensions, a slanted roof and an open bottom. Aerial boxes were hung in well shaded locations approximately 1-2 meters high in trees surrounding the Mountain Research Station. I intended for these aerial nesting boxes to simulate the eave of a building, which is common nesting location (Akre *et al.* 1976). Again, these boxes were monitored throughout the summer months for wasp activity as well as for nest mortality if prior to the collection of all the boxes in early September.

Beginning in mid-July 2003, once nests had grown large enough to be easily spotted, I collected colonies opportunistically. Public notification was accomplished via flyers which were distributed from Wondervu, Colorado to Estes Park, Colorado in a variety of locations. Additionally, an article was published in the *Mountain Ear* (a small newspaper servicing the same area) detailing the research being conducted and who to contact if people had yellow jacket nests on their property. When nests were located, I removed them upon property owner's request or allowed them to persist as long as the wasps did not become a nuisance to the property owner.

After collection the boxes were placed in a standard freezer until the colony died. I collected the natural opportunistically found nests by asphyxiating the entire

colony with compressed carbon dioxide gas. Once in the laboratory all nests and individuals were kept at -30C to preserve the specimens. The following quantitative nest data were recorded; overall size, number of sheaths, number of combs, number of cells per comb and the number/sex of individuals.

Laboratory methods

DNA was extracted from the thorax of queens, workers and male reproductives using Qiagen animal kits. Three microsatellite primers (Rufa 5, 13, 15) previously developed using *Vespula rufa* as described in Thorén *et al.* (1995) and published by Foster *et al.* (2001) were used to assay the genotypes of all individual (Table 2). To our knowledge, this was the first time these primers were used with *D. arenaria*, so I optimized polymerase chain reaction (PCR) procedures for each microsatellite.

Table 2. Primer sequences (Thorén *et al.* 1995 and published by Foster *et al.* 2001)

Rufa 5	F	GAGGCAAATTTTACGACGTAGG
	R	CTGCCAATCGCATGTCTG
Rufa 13	F	GATCTGTGTACGTAATTCTCTCC
	R	GAAAATCGGGAACGATG
Rufa 15	F	GATCAGAAATCTGATTAAGTCGAG
	R	GGTCGATTCGTTTGAAAATAG

To visualize microsatellites using fluorescent techniques, the forward primers of each microsatellite were labeled with a TET (Integrated DNA Technologies, INC.). PCRs were conducted in a final volume of 25 μ l containing 1 μ l of genomic DNA and 0.2 μ l *Taq* DNA polymerase (Promega), and a final concentration of 8 μ M dNTPs, 1 μ M and 10 μ M of the labeled forward PCR primer and reverse PCR primer,

respectively, and 2.5 μ l of 10x Promega Buffer. The final concentration of Magnesium Chloride for Rufa 5 and Rufa 13 was 2.0 μ M and for Rufa 15 it was 1.2 μ M. The PCR cycling profiles for the three primers began with an initial denaturation at 94 °C for 1 min, and then proceeded with 25 cycles of 94 °C for 30 s, 44 °C for 30 s (Rufa 5 and 15) or 46 °C for 30 s (Rufa 13) and 72 °C for 45 s, followed by a final extension of 72 °C for 5 min. PCR products were then electrophoresed on an MJ Research, Base Station 51 DNA Fragment Analyzer and analyzed with Cartographer software at the University of Colorado, Ecology and Evolutionary Biology Sequencing Laboratory. Gels were scored twice independently (by two people) and results were compared, with discrepancies sometimes being reconciled by re-conducting the genotyping entirely (Tóth *et al.* 2002a, Strassmann *et al.* 2003).

Statistical analysis

Analysis of the genotyping data was accomplished using the program *Relatedness 4.2 and 5.0* (Goodnight and Queller, 2000). This software calculated the regression relatedness between individuals. *Relatedness 4.2* was used specifically to calculate the inbreeding coefficient. Expected heterozygosity was calculated with the following equation:

$$H_e = 1 - \sum p_i^2, \text{ summed over each allele, where } p \text{ is the allele frequency.}$$

Effective paternity was estimated after Starr (1984)

$$M_e = \frac{n}{\sum (\sum_i p_i^2)_j},$$

where p_i is the proportional contribution of the i th male in the j th nest for n nests.

To determine which males were either queen or worker offspring, I first used the exclusion method. If a male has one of the queen's two alleles, he was counted as a queen's son regardless of whether a worker shares that same allele. Conversely, a male would only be counted as a worker's son if he carried an allele which was only present in the workers. Thus, with this exclusion method some males could be erroneously counted as the queen's offspring.

An alternate method to estimate the proportion of males produced by the queen or the workers is the likelihood method (Tóth *et al.* 2002) which is a modified version of that used by Arévalo *et al.* (1998), designed originally for multiple queen species. For each colony I used the worker and male genotypes to infer the queen's genotype. Let Q be the fraction of males that come from the queens. I tested various hypothetical values of Q to see which has the highest likelihood of having produced the observed male data (in practice, I tested all values between 0 and 1 at increments of 0.02). The likelihood, L , for any hypothetical value of Q is calculated as

$$L = K \prod_{\text{males}} [Q \prod_{\text{loci}} f_{qi} + (1 - Q) \prod_{\text{loci}} f_{wi}]$$

K is a multinomial constant that never has to be calculated because it multiplies all L s by the same value, and cancels out any comparisons. For each male allele considered in turn, f_{qi} and f_{wi} are the frequencies of that allele in the queen and in the workers.

A complication arises when workers are all heterozygous, because it cannot be determined which allele came from the queen and which from her mate. In this case, I average the likelihoods of both possibilities (AAxB and BBxA), weighted by their likelihoods of occurring using an Excel spreadsheet with embedded calculations from Dr. David Queller of Rice University. This spreadsheet allows for the input of allele

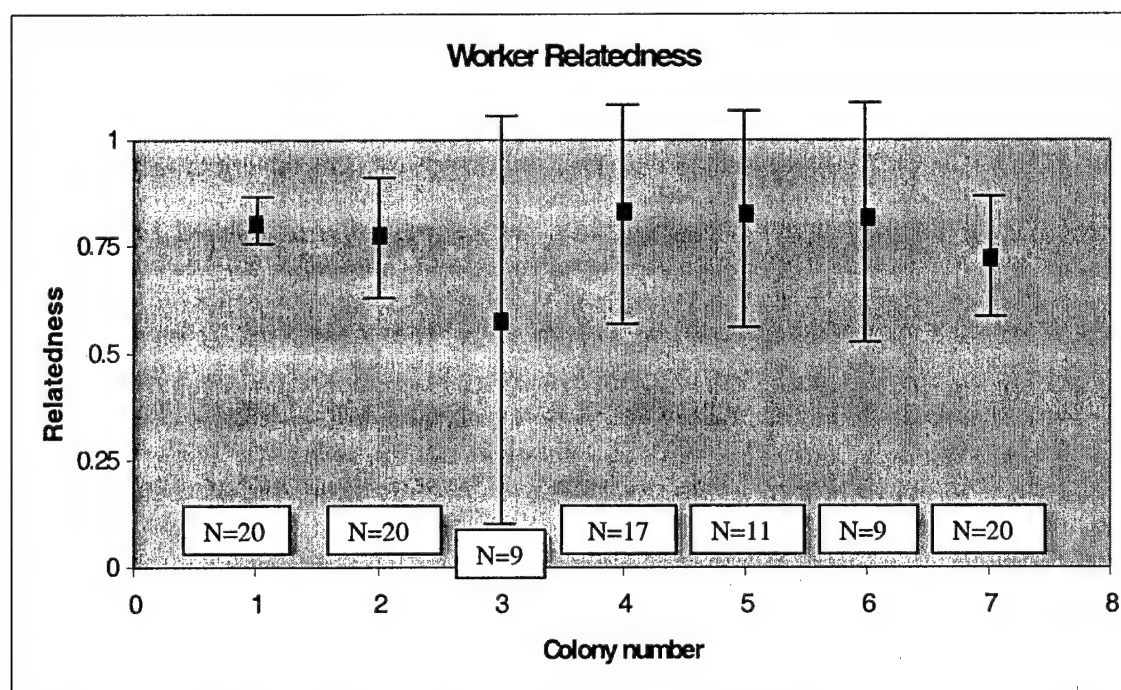
frequencies, queen's and mate's genotype, and reproductive male genotypes and then calculates the likelihoods. Calculating the two likelihoods separately in this way, and then averaging, is the best method because one of the two matings is correct and it applies to all offspring; it makes no sense to arbitrarily assume one mating for some offspring and the other mating for the rest.

Results

Within-colony relatedness

Mean worker-worker relatedness was near Hamilton's (1964) predicted value of 0.75 for single-mated haplodiploid queens in an outbred population (Table 3, Figure 2). The 95% confidence intervals ranged from 0.05 to 0.45 and were highly influenced by sample size.

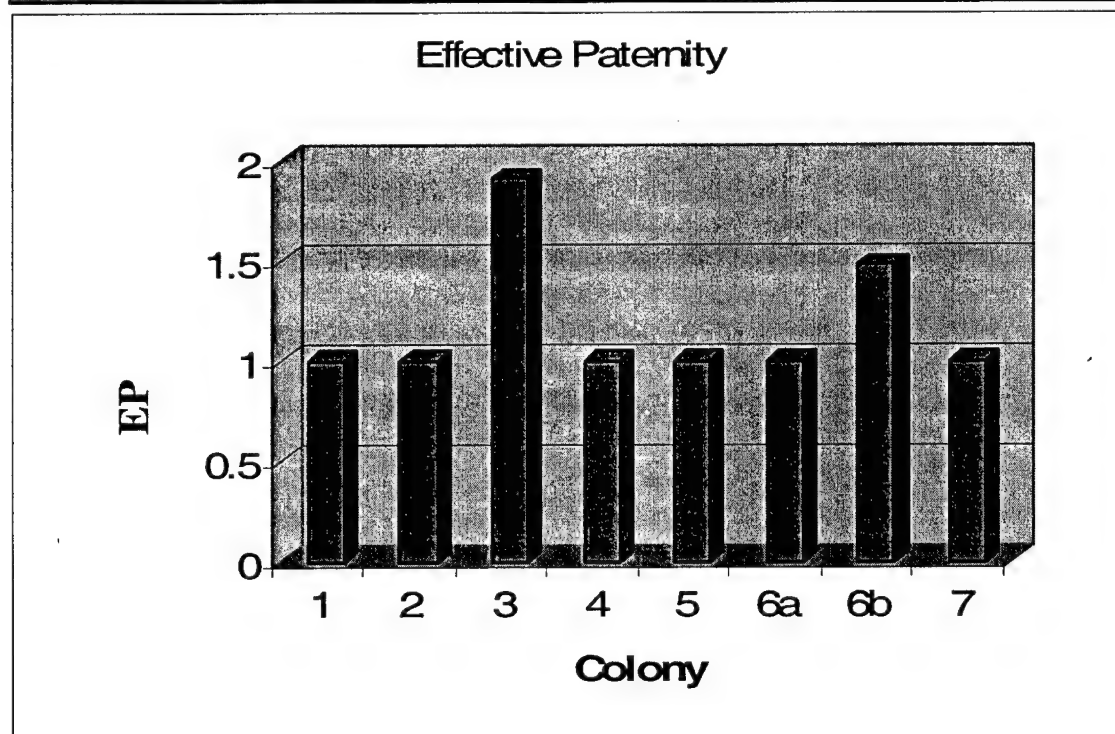
Figure 2. Mean relatedness estimates among workers in *D. arenaria* colonies (plus or minus 95% CI). The line across the graph indicates expected relatedness based upon a single mating. Number of workers sampled for each colony appears in the box above the colony number.



Effective paternity - M_e

The mean effective paternity (M_e) within this population was 1.13. I deduced the queen's and her mate's genotype from worker genotypes in all cases except in colony five, in which the queen was still present at the time of collection. In five of the seven colonies, once the queen's genotype was deduced, the array of worker genotypes could be explained by single male genotype, suggesting a single mating by the queen and an effective paternity of 1.0. Worker genotypes in colony six could be explained either by a single mating by the queen ($M_e = 1.0$) or a double mating by the queen which yielded an M_e of 1.48. In this case, I believe a single mating to be the most parsimonious explanation based upon the worker and male genotypes. Colony three worker genotypes indicated up to 3 matings by the queen, an effective paternity of 1.91, and mean worker relatedness of 0.58, much lower than any other colony (Table 3, Figure 2).

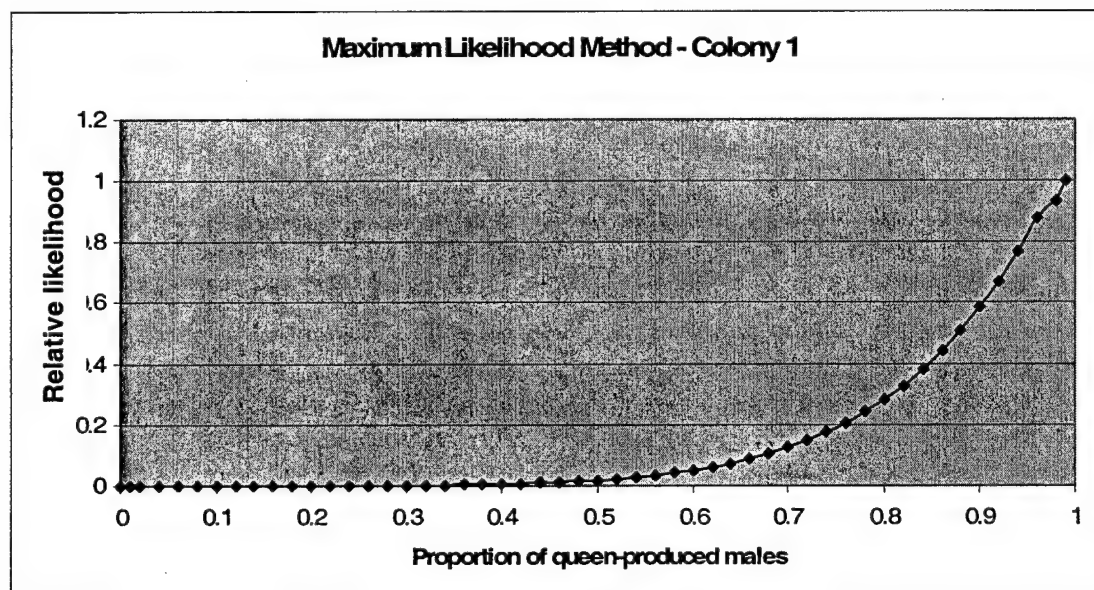
Figure 3. Effective paternity by colony



Male production

In each of the four colonies that contained males, the queen produced an overwhelming majority of the males, on average 91.5%. Both the exclusion method and the maximum likelihood method of determining the origin of the males suggest that in most colonies the males originated from queen-laid eggs. Colony seven had the largest percentage of worker produced males, 20% by the exclusion method or 24% by the maximum likelihood method. Other colonies ranged from 0 to 8% worker produced males.

Figure 4. Maximum likelihood graphs. The sharper the peak, the greater the distinction between queen and worker produced male.



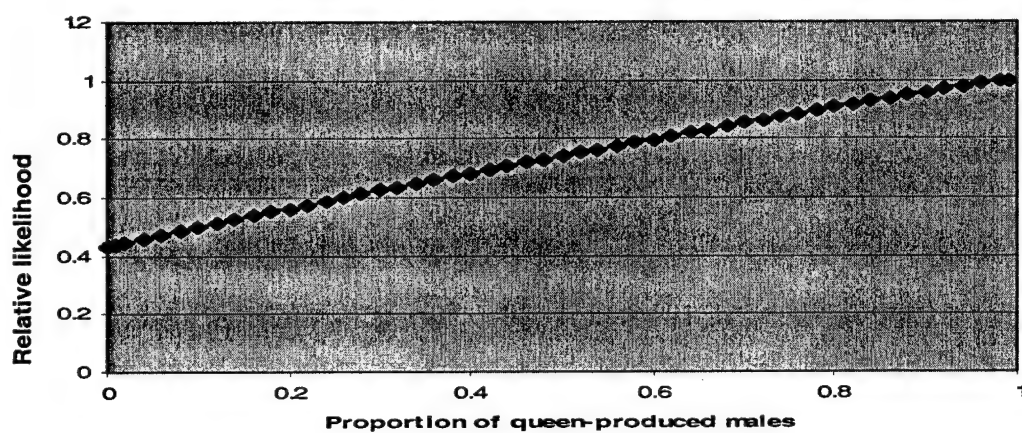
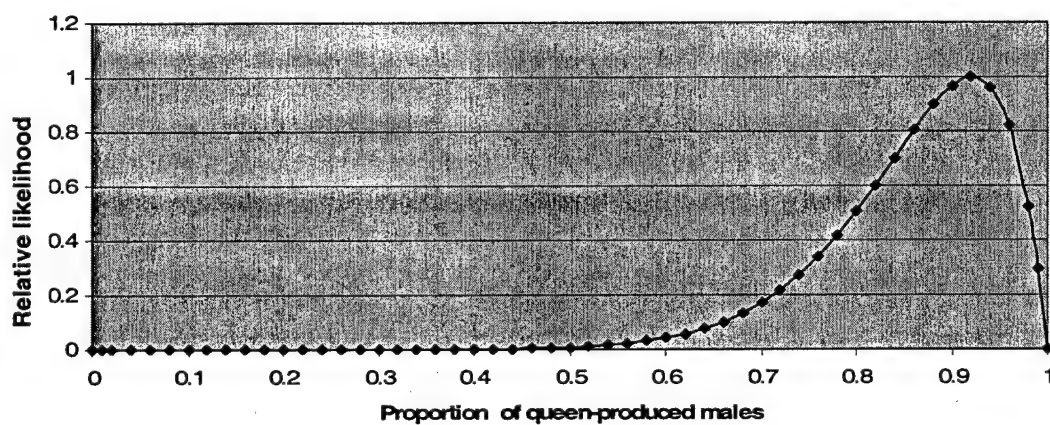
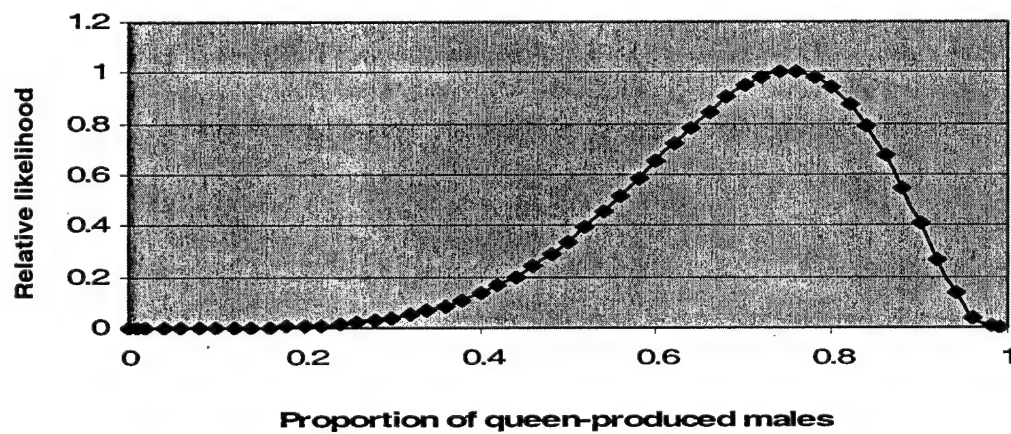
Maximum Likelihood Method - Colony 2**Maximum Likelihood Method - Colony 6****Maximum Likelihood Method - Colony 7**

Table 3. Summary data for seven colonies of *D. arenaria*.

Colony	No. of workers analyzed	Mean worker/ worker relatedness	Effective paternity M_e	No. of males analyzed	Exclusion		Maximum Likelihood	
					% Worker produced males	% Queen produced males	% Worker produced males	% Queen produced males
1	20	0.8052	1.0	8	0	100	2	98
2	20	0.7801	1.0	2	0	100	-	100
3	9	0.5778	1.91	0	-	-	-	-
4	17	0.8319	1.0	0	-	-	-	-
5	11	0.8299	1.0	0	-	-	-	-
6	9	0.8225	1.0/1.48*	17	0	100	8	92
7	20	0.7252	1.0	10	20	80	24	76

*First estimate assumes queen mated once, second estimate assumes queen mated twice. The data were not adequate to discriminate between these two hypotheses.

Queen presence and reproductive stage

Table 4 summarizes the status of the queen in the colonies sampled. Of the seven colonies collected, I found a queen in only one colony. This colony actually had two queens because the nest had been parasitized by *D. arctica*, which was present as well.

Table 4. Queen presence and reproductive stage

Colony	Queen present	Producing reproductives	Parasitized
1	No	Yes	No
2*	No	Yes	No
3	No	No	Yes
4	No	No†	No
5	Yes	No	Yes
6	No	Yes	No
7	No	Yes	No

*indicates the nest was sprayed with insecticide prior to collection

†indicates no male eggs or larvae found, but female reproductive could be present

Allelic diversity

At the locus Rufa 5, there were 16 different alleles ranging from 158 to 204 base pairs in length. Rufa 13 had 9 alleles starting at 169 base pairs and ranging to 187 base pairs. Rufa 15's 11 alleles started at 115 base pairs and ranged to 148. Allele frequencies for each locus are shown in Table 5.

Table 5. Allele length and frequency for each locus

Rufa 5		Rufa 13		Rufa 15	
length	Frequency	Length	Frequency	length	Frequency
160	0.14286	170	0.05	115	0.17324
162	0.02381	172	0.10317	117	0.05556
166	0.07143	174	0.07983	119	0.05
168	0.03571	176	0.07143	121	0.0873
170	0.02857	178	0.24167	125	0.11345
172	0.05386	180	0.15754	127	0.04545
174	0.02597	182	0.11064	131	0.09643
178	0.10754	184	0.07143	133	0.22024
182	0.11071	186	0.11429	137	0.07143
184	0.07103			139	0.04762
186	0.04762			147	0.03929
188	0.06303				
194	0.11905				
200	0.05873				
202	0.00794				
204	0.03214				

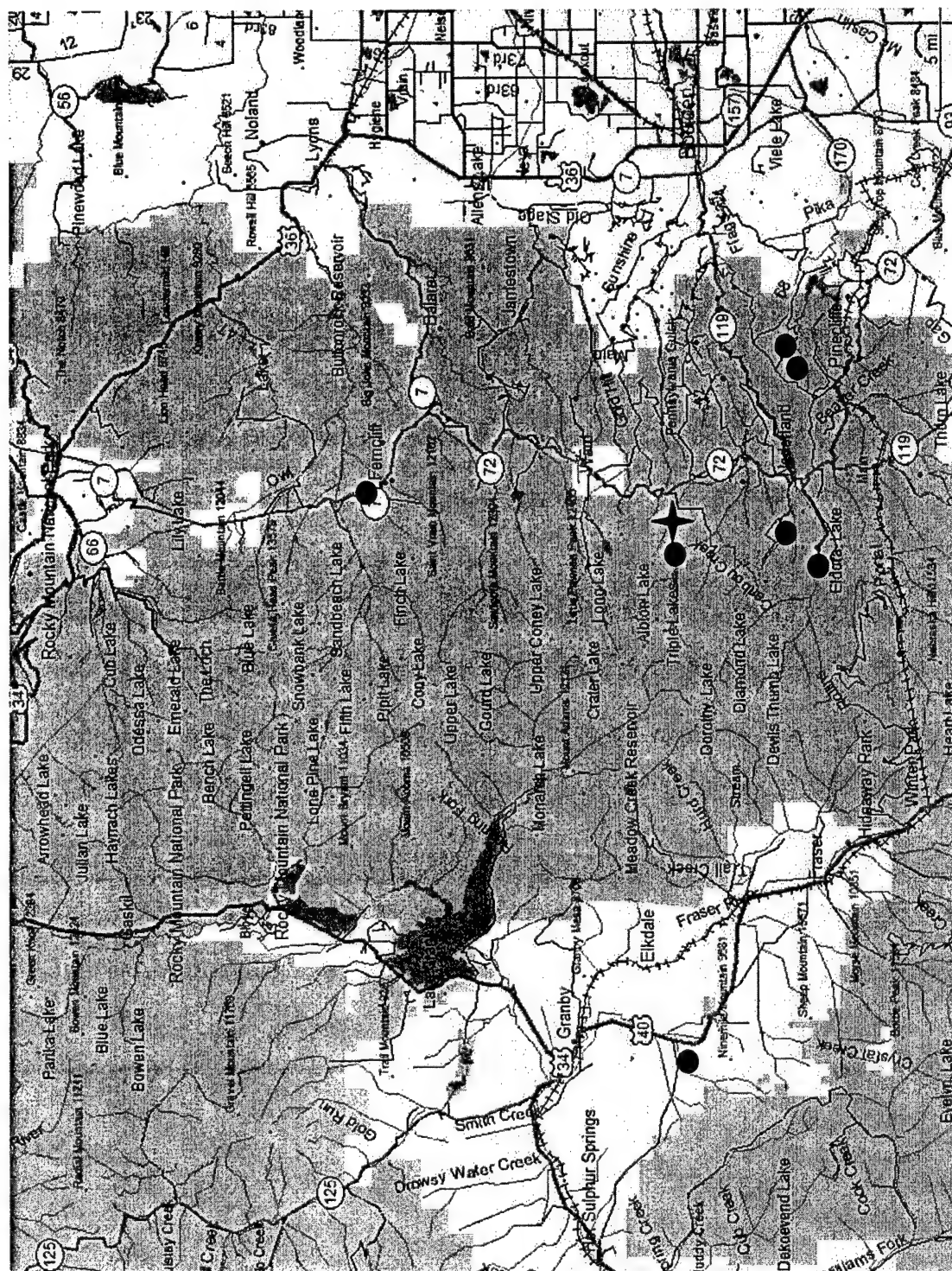
Heterozygosity and inbreeding coefficient

Expected heterozygosity was calculated from allele frequencies at each locus (Table 6). Based on allelic frequencies, population inbreeding (F_{is}) was also calculated. An F_{is} of 0.05 indicates only a slight degree of inbreeding.

Table 6. Heterozygosity for each locus and inbreeding coefficient (F_{is})

	<u>Rufa 5</u>	<u>Rufa 13</u>	<u>Rufaa 15</u>	<u>Population F_{is}</u>
Expected Heterozygosity	0.9144	0.8643	0.8751	0.0524
Observed Heterozygosity	1.0	0.8390	0.8390	

Figure 5. Nest locations indicated by red circles



Discussion

Effective paternity across the population was low (1.13) and similar to that found in a European population of *D. arenaria* (1.09) (Boomsma and Ratnieks, unpublished, cited in Foster and Ratnieks 2001). Mean worker-worker relatedness (0.77) was close to Hamilton's predicted worker-worker relatedness of 0.75 in outbred species, and was consistent with the five other *Dolichovespula* species for which data is available (0.69) (Foster *et al.* 2001). The queens of colony six and three had multiple matings, however effective paternities calculated at 1.48 and 1.91 respectively, which are still below the threshold of 2.0 established by (Boomsma and Ratnieks 1996, Foster and Ratnieks 2001c) for multiple mating; any effective paternity above two significantly impacts the worker-worker relatedness.

The conclusion that *Dolichovespula* species mate singly is supported by my data. Therefore, a conflict over male production between workers and the queen should exist within *Dolichovespula* colonies (Hamilton 1964, Woyciechowki and Lomnicki 1987, Foster *et al.* 2001). Foster *et al.* (2001) found a range of male production by workers from 2.6 to 34.6% of total male production with an average of 15% across their five species of *Dolichovespula*. My study population of *D. arenaria* had a smaller range of worker-produced males, 0 to 24% with the average being 8.5%. A true outlier in the clade is *Dolichovespula saxonica* with a mean effective paternity of 1.35 and mean worker male production of 34.6% (Foster *et al.* 2001). The queen clearly lays the majority of the male eggs in all *Dolichovespula* species for which data are available. Additionally, the four colonies in this study

which produced males each lacked a queen at the time of collection, and yet, the workers failed to capitalize on this opportunity.

The practice of monandry by *D. arenaria* is consistent with high levels of intracolony relatedness among workers. Therefore, eusociality in *D. arenaria* can be explained by kin selection theory. By mating just once, *D. arenaria* sacrifices any potential benefit gained from polyandry. These benefits could include additional sperm and genetically variable brood. As an annual species, a queen should have enough sperm from a single mating to lay the required number of workers (500-1000) for colony growth and reproduction (Greene 1991). The potential benefits derived from a genetically diverse brood include increased task specialization, disease resistance, decreased chance of producing sterile diploid males and a reduction in queen-worker conflict over sex ratios and male production. Perhaps the benefits from monandry, which largely come from high worker-worker relatedness, outweigh any of the potential benefits of polyandry.

The queen seems to be in control of male reproduction, whether present or not, and this translates into a low level of queen-worker conflict over male production. How does the queen maintain control over male production? Pheromonal control by the queen has been the traditional explanation, although some have criticized pheromonal control as being evolutionarily unstable because workers will be selected to not respond to the pheromone (Foster *et al.* 2001). An alternative to pheromonal control, or most likely in addition to, is worker policing (Foster and Ratnieks 2001b). Under the scenario of single mating, a worker is more related to the queen's sons than the sons produced by other workers. So workers remove eggs that

were laid by other workers and allow the queen to dominate male production.

Typically there are only a few workers with active ovarioles that can lay mature eggs. In four other *Dolichovespula* species it was found that a typical colony will only have four to eight reproductive workers (Foster *et al.* 2001). So, if only a few workers produce eggs and worker policing limits the number of worker eggs that actually mature, there should be a low success rate in worker reproduction and such behavior should not spread through a population. Worker policing could balance with queen pheromonal control to create an evolutionarily stable system.

All of the four colonies analyzed for male production were queenless at the time of collection; this introduces the concept of matricide. The possibility of simply missing the queen at the time of collection are extremely low because all colonies were collected after sunset, thus all foragers should have returned to the nest and secondly a queen seldom, if ever, forages after the eclosion of her first worker brood. Again, because of the higher worker relatedness to their own sons as compared to the queen's sons, matricide is predicted in singly mated species (Ratnieks 1988, Foster *et al.* 2001, Foster and Ratnieks 2001a). In theory, the workers gain direct fitness via male production after killing the mother queen. Oddly, only one of four colonies had a significant percentage of worker male production; this could be explained by the timing of matricide with respect to the time of collection. If under pheromonal control, it typically takes 10 to 14 days for workers to develop fully functioning ovarioles once released from the queen's pheromone (Spradbery 1973), thus the queen may have not been dead long enough to allow for ovariole development in the workers. These 14 days added to the 30 days it takes for an egg to develop into an

adult equals six weeks before eclosion of the first worker produced males after the queen's death. So, I may have simply missed the period of highest worker male production. However, the timing of matricide is critical for the workers to gain any reproductive fitness. If matricide occurs late in the season, eclosion of worker produced males may not occur prior to the first frost or as a minimum, these males may miss the peak of virgin queen emergence from nests which would result in a low chance for reproduction. In this study 86% of all nests collected were queenless and Foster and Ratnieks (2001a) cited unpublished data that found 54% of nest queenless. Thus, matricide remains a strong possibility within *D. arenaria*, although more is required to determine if matricide increases worker inclusive fitness.

The presence of an obligate parasitic wasp species (*D. arctica*) likely led to the demise of one queen. Although this obligate parasite is thought to kill the natal queen early in the growing season, in one nest it was clearly not the case as the nest was collected on 27 August and both queens were still present. In the other nest which had been parasitized, the *D. arenaria* queen was absent. Three of the seven colonies collected contained no reproductives; two of these were parasitized which may indicate the handicapping of the host colony by the parasite. I attempted to find male pupae, larvae or eggs in the last of the queenless colonies (colony four) but found none. This colony might of had some female reproductives in the egg or larval stages which were laid prior to the queen's demise, but this colony was destined to produce 100% worker laid males. This of course is dependent on some workers having active ovarioles. Having not found any haploid eggs, I suspect this is a case of

matricide and the workers have not been free of the queen's controlling pheromones long enough to produce viable eggs.

In the larger context of the Hymenoptera my data fall within a wide spectrum of effective paternity and the resolution of queen-worker conflict over male production. Even within *Dolichovespula* there is quite a range, mainly due to *D. saxonica*. Foster and Ratnieks (2000) found that *D. saxonica* colonies mated multiply and demonstrate worker policing, while singly mated colonies do not. Worker policing has also been found in the European hornet *Vespa crabro* ($M_e = 1.11$) (Foster *et al.* 1999) and in the common wasp *Vespula vulgaris* ($M_e = 1.90$) (Foster and Ratnieks 2001). This trend holds true for honey bees, although they have a much higher effective paternity ($M_e > 5.0$, Tarpy and Nielsen 2002). Bumble bees have a low effective paternity and workers produce over 20 percent of the males, suggesting a lack of worker policing. Stingless bees also have a low effective paternity but a wide range of percent worker produced males (Tóth *et al.* 2002a, Tóth *et al.* 2002b, Paxton *et al.* 2003, Tóth *et al.* 2003). There is variability among ants as well: for fungus growing ants that mate singly -- in colonies with a queen, she laid 100% of the male workers but in queenless colonies workers produce the males (behavior data suggest queen control not worker policing) (Villesen and Boomsma 2003), but worker policing occurs in three species of queenless ants where it may also have evolved contrary to relatedness predictions (Gobin *et al.* 1999). So in some cases workers are replacing their more-related nephews by less-related brothers.

This research adds to present knowledge of yellow jacket effective paternity and male production but an analysis of more nests would bring better resolution to my

findings with regard to *D. arenaria*. These results suggest the queens may truly be in control of the genetic structure of this population because she is producing both the male and female reproductives and thus maintaining eusociality of this species. An analysis of ovariole activation of the workers within this study would clarify the degree of queen-worker conflict over male production. If worker ovariole activation is low then a conclusion of little to no conflict in the colony would be supported, while a high rate of ovariole activation would be evidence for a higher level of conflict. To my knowledge, within-nest behavioral research has not been accomplished with *D. arenaria*. A better understanding of worker-worker and worker-queen interactions would provide an improved context in which to overlay what we already understand about effective paternity and male production.

References

- Akre RK, Greene A, MacDonald JF, Landolt, PJ, Davis HG (1980) *Yellowjackets of America North of Mexico*. U.S. Dept. of Ag, Agriculture Handbook No. 552.
- Arévalo E, Strassmann JE, Queller DC (1998) Conflicts of interests in social insects: male production in two species of *Polistes*. *Evolution*, **53**, 797-805.
- Blanchetota A (1991) Genetic relatedness in honeybees as established by DNA fingerprinting. *Journal of Heredity* 82 (5), 391-396.
- Boomsma JJ and Ratnieks LW (1996) Paternity in eusocial Hymenoptera. *Philosophical transactions of the Royal Society of London B* **351**, 947-975.
- Brown MJF, Schmid-Hempel R, Schmid-Hempel P (2003) Queen-controlled sex ratios and worker reproduction in the bumble bee *Bombus hypnorum*, as revealed by microsatellites. *Molecular Ecology* **12**, 1599-1605.
- Crespi BJ (1992) Eusociality in Australian gall thrips. *Nature*, **359**, 724-726.
- Darwin C (1859) On the origin of species. 1st edition. Harvard University Press, Cambridge, Mass.
- Fewell JH, Page RE (1993) Genotypic variation in foraging responses to environmental stimuli by honey bees, *Apis mellifera*. *Experientia* **19**, 1106-1112.
- Field J, Solís CR, Queller DC, Strassmann JE (1998) Social and genetic structure of paper wasp cofoundress associations: tests of reproductive skew. *American Naturalist* **151**, 545-563.
- Fjerdingstad EJ, Gertsch PJ, Keller L (2002) Why do some social insect queens mate with several males? Testing the sex-ratio manipulation hypothesis in *Lasius niger*. *Evolution* **56** (3), 553-562.
- Foster KR, Seppä P, Ratnieks FLW, Thoren PA (1999) Low paternity in the hornet *Vespa crapro* indicates that multiple mating by queens is derived in vespine wasps. *Behavioral Ecology and Sociobiology* **46**, 252-257.
- Foster KR, Ratnieks FLW, Raybould AF (2000) Do hornets have zombie workers? *Molecular Ecology* **9**, 735-742.
- Foster KR, Ratnieks FLW, Gyllenstrand N, Thorén PA (2001) Colony kin structure and male production in *Dolichovespula* wasps. *Molecular Ecology* **10**, 1003-1010.

- Foster KR, Ratnieks FLW (2001a) Paternity, reproduction and conflict in vespine wasps: a model system for testing kin selection predictions. *Behavioral Ecology and Sociobiology* **50**, 1-8.
- Foster KR, Ratnieks FLW (2001b) Convergent evolution of worker policing by egg eating in the honey bee and common wasp. *Philosophical transactions of the Royal Society of London B* **268**, 169-174.
- Foster KR, Ratnieks FLW (2001c) The effect of sex-allocation biasing on the evolution of worker policing in hymenopteran societies. *American Naturalist* **158**(6), 615-623.
- Gobin B, Billen J, and Peeters C (1999) Policing behaviour towards virgin egg layers in a polygynous ponerine ant. *Animal Behaviour* **58**:1117-1122.
- Goodnight KF, Queller DC (2000) Relatedness 5.0 and 4.2
<http://gsoftnet.us/RLAT.hqx>
- Greene A, Akre RD, Landolt PJ (1976) The aerial yellowjacket, *Dolichovespula arenaria*: nesting biology, reproductive production, and behavior (Hymenoptera, Vespidae). *Melanderia* **26**, 1-34.
- Greene A (1991) *Dolichovespula* and *Vespula*. In: *The social biology of wasps* (eds Ross KG and Matthews RW), pp 263-305. Cornell University, Ithaca, NY.
- Hamilton WD (1964) The genetical evolution of social behavior. *Journal of Theoretical Biology* **7**, 1-52.
- Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. *Biological Review* **75**, 21-64.
- Page RE (1986) Sperm utilization in social insects. *Annual Review of Entomology* **31**, 297-320.
- Paxton RJ, Ayasse M, Field J, Soro A (2002) Complex sociogenetic organization and reproductive skew in a primitively eusocial sweat bee, *Lasioglossum malachurum*, as revealed by microsatellites. *Molecular Ecology* **11**, 2405-2416.
- Paxton RJ, Bego LR, Shah MM, Mateus S (2003) Low mating frequency of queens in the stingless bee *Scaptotrigona postica*¹ and worker maternity of males. *Behavioral Ecology and Sociobiology* **53**, 174-181.
- Peters JM, Queller DC, Strassmann JE, Solís CR (1995) Maternity assignment and Queen replacement in social wasps. *Philosophical transactions of the Royal Society of London B* **260**, 7-12.

- Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *American Naturalist*, **132**, 217-236.
- Ratnieks FLW, Visscher PK (1989) Worker policing in the honeybee. *Nature* **342**, 796-797.
- Ratnieks FLW, Reeve HK (1992) Conflict in single queen Hymenopteran societies: structure of conflict and processes that reduce conflict in advanced eusocial species. *Journal of Theoretical Biology* **158**, 33-65.
- Ratnieks FLW, Monnin T, Foster KR (2001) Inclusive fitness theory: novel predictions and tests in eusocial Hymenoptera. *Annales Zoologici Fennici* **38**, 201-214.
- Romoser WS and Stoffolano JG (1998) *The Science of Entomology*, 4th edition. Wm. C. Brown Publishers, Dubuque, Iowa.
- Ross KG (1985) Aspects of worker reproduction in four social wasp species (Insecta: Hymenoptera: Vespidae). *Journal of Zoology. Series A: Proceedings of the Zoological Society of London* **205**, 411-424.
- Ross KG (1986) Kin selection and the problem of sperm utilization in social insects. *Nature* **323**, 798-800.
- Seppä P, Queller DC and Strassmann JE (2002) Reproduction in foundress association of the social wasp, *Polistes carolina*: conventions, competition and skew. *Behavioral Ecology* Vol **13** No **4**, 531-542.
- Schmid-Hempel P, Crozier RH (1999) Polyandry versus polygyny versus parasites. *Philosophical transactions of the Royal Society of London B* **354**, 507-515.
- Schmid-Hempel R, Schmid-Hempel P (2000) Female mating frequencies in *Bombus* spp. from Central Europe. *Insectes Sociaux* **47**, 36-41.
- Spradbery JP (1971) Seasonal changes in the population structure of wasp colonies (Hymenoptera: Vespidae). *Journal of Animal Ecology* **40**, 501-523.
- Spradbery JP (1973) *Wasps*. University of Washington Press, Seattle, WA.
- Starr CK (1984) Sperm competition, kinship and sociality in the aculeate Hymenoptera. In: *Sperm competition and the evolution of animal mating systems* (ed. Smith RL), pp 427-464. Academic Press, Orlando, FL.
- Strassmann JE, Hughes CR, Queller DC, Turillazzi S, Cervo R, Davis SK, Goodnight KF (1989) Genetic relatedness in primitively eusocial wasps. *Nature* **342**, 268-

270.

- Strassmann JE (2001) The rarity of multiple mating by females in the social Hymenoptera. *Insectes Sociaux* **48**, 1-13.
- Strassmann JE, Nguyen JS, Arévalo E, Cervo R, Zacchi F, Turillazzi S, Queller DC (2003) Worker interest and male production in *Polistes gallicus*, a Mediterranean social wasp. *Journal of Evolutionary Biology* **16**, 254-259.
- Tarpy DR, Nielsen DL (2002) Sampling error, effective paternity and estimating the genetic structure of honey bee colonies (Hymenoptera: Apidae). *Annals of the Entomological Society of America* **95**, 513-528.
- Thorén PA, Paxton RJ, Estoup A (1995) Unusually high frequency of (CT)_n and (GT)_n microsatellite loci in a yellowjacket wasp, *Vespula rufa* (L.) (Hymenoptera, Vespidae). *Insect Molecular Biology*, **4**, 141-148.
- Tóth E, Strassmann JE, Nogueira-Neto P, Imperatriz-Fonseca VL, Queller DC (2002a) Male production in stingless bees: variable outcomes of queen-worker conflict. *Molecular Ecology* **11**, 2661-2667.
- Tóth E, Queller DC, Imperatriz-Fonseca VL, Strassmann JE (2002b) Genetic and behavioral conflict over male production between workers and queens in the stingless bee *Paratrigona subnuda*. *Behavioral Ecology and Sociobiology* **53**, 1-8.
- Tóth E, Strassmann JE, Imperatriz-Fonseca VL, Queller DC (2003) Queens, not workers, produce the males in the stingless bee *Schwarziana quadripunctata quadripunctata*. *Animal Behaviour* **66**, 359-368.
- Trivers RL, Hare H (1976) Haplodiploidy and the evolution of social insects. *Science* **191**, 249-263.
- Villesen P and Boomsma JJ (2003) Patterns of male parentage in the fungus-growing ants. *Behavioral Ecology and Sociobiology* **53** (4): 246-253 MAR 2003
- Wilson EO (2000) Sociobiology, the new synthesis. Harvard University Press. Cambridge, Massachusetts.
- Woyciechowki M, Lomnicki A (1987) Multiple mating of queens and the sterility of workers among eusocial Hymenoptera. *Journal of Theoretical Biology* **128**, 317-327.